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MECHANISM OF ALDOSE REDUCTASE MEDIATED INTESTINAL MUCOSAL INJURY IN ACUTE HYPOXIC YOUNG RATS BY REGULATING MITOCHONDRIAL FUNCTION

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ABSTRACT

Objective: *To explore the mechanism of aldose reductase mediated intestinal mucosal injury in acute hypoxic young rats by regulating mitochondrial function.*

Methods: *Thirty SD rats were randomly divided into sham operation group, model group and eparltast group, with 10 rats in each group. Intestinal ischemia-reperfusion models were established in both model group and AR inhibitor group. Epalrestat group was injected 10mg/kg epalrestat intravenously 10 minutes before modeling. In the sham operation group, only the superior mesenteric artery was exposed and isolated and sutured. He staining was used to detect the pathological changes of small intestine tissues of rats in each group, and the degree of injury was evaluated according to Aurelie Le mandat Schultz pathological score. The expression levels of Bcl-2, Bax mRNA and protein in small intestine tissues of rats in each group were detected by fluorescence quantitative PCR and Western blot. The contents of lactic acid and MDA in rat mucosal tissue were detected by spectrophotometer, the level of MPO was detected by colorimetry, the apoptosis rate was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), and the mitochondrial transmembrane potential of intestinal mucosal cells was detected.*

Results: *the pathological scores of rats in the model group and eparltast group were significantly higher than those in the sham operation group ($P<0.05$); The pathological score of rats in eparltast group was significantly higher than that in model group ($P<0.05$). Lactic acid, MDA and MPO in mucosal tissue of rats in model group and eparltast group were significantly higher than those in sham operation group ($P<0.05$); Lactic acid, MDA and MPO in mucosal tissue of rats in eparltast group were significantly higher than those in model group ($P<0.05$). The apoptosis rate of mucosal tissue in model group and eparltast group was significantly higher than that in sham operation group ($P<0.05$); The apoptosis rate of mucosal tissue in eparltast group was significantly higher than that in model group ($P<0.05$). The Bcl-2 mRNA and protein in the mucosa of rats in the model group and eparltast group were significantly lower than those in the sham operation group ($P<0.05$); Bcl-2 mRNA and protein in mucosal tissue of rats in eparltast group were significantly lower than those in model group ($P<0.05$). Bax mRNA and protein in mucosal tissue of rats in model group and eparltast group were significantly higher than those in sham operation group ($P<0.05$); Bax mRNA and protein in mucosal tissue of rats in eparltast group were significantly higher than those in model group ($P<0.05$). The mitochondrial membrane potential of rat mucosal tissue cells in the model group and eparltast group were significantly lower than that in the sham operation group ($P<0.05$); The mitochondrial membrane potential of mucosal tissue cells in eparltast group was significantly lower than that in model group ($P<0.05$).*

Conclusion: *aldose reductase plays a role in mediating intestinal mucosal injury in young rats with acute hypoxia, and its mechanism may be achieved by regulating mitochondrial function.*

Keywords: *Aldose reductase, mitochondria, intestinal mucosal injury, mechanism.*

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Introduction

Intestinal ischemia-reperfusion in children is commonly seen in children with intestinal diseases such as necrotizing enterocolitis, strangulation intestinal obstruction and volvulus after blood flow restoration and perfusion after treatment[1]. Based on the intestinal metabolism active, specific hairpin mucosal vascular structure, after ischemia and reperfusion intestinal injury is particularly common[2], which not only cause intestinal tissue damage, at the same time, between intestinal mucosal barrier function damage can lead to toxins and even bacteria, and under the stimulation of proinflammatory factors can cause a cascade of inflammation, this will cause multiple organ insufficiency syndrome, a serious threat to children's life and health[3]. It is important to explore the mechanism of ischemia-reperfusion

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intestinal injury and prevent intestinal mucosa injury. Previous studies found that aldose reductase inhibited the intestinal mucosal injury[4], and existing studies confirmed that mitochondrial pathway is involved in regulating ischemia-reperfusion intestinal mucosal injury[5], and aldose reductase improved mitochondrial function to protect cerebral ischemia-reperfusion injury[6], so this study intends to explore whether aldose reductase mediated the mechanism of intestinal mucosal injury by regulating mitochondrial function.

Materials and methods

Experimental materials

Thirty healthy SPF SD rats, aged 6 days, were purchased from Guangdong Medical Laboratory Animal Center, batch number: SCXK (Guangdong) 2021-0014, and were fed freely by female rats for 7 days before the experiment.

Methods

- Thirty SD rats were randomly divided into sham operation group, model group and Eparltast group, with 10 rats in each group. Rats in model group and Eparltast group were anesthetized with uratan (1g/kg) and fixed on the operating plate. The superior mesenteric artery was isolated and clipped with a arteriolar clip. The color of small intestine tissue changed from bright red to dark red with no pulsatile superior mesenteric artery. After 60 minutes, the arterial clamp was removed and blood supply was restored, and the color of small intestine tissue changed from dark red to bright red with pulsatile superior mesenteric artery and perfusion. The intestinal ischemia-reperfusion model was successfully constructed. Eparltast group: 10mg/kg Epalrestat was injected intravenically 10min before modeling. In the sham group, only the superior mesenteric artery was exposed and isolated and sutured.
- Another 2cm small intestinal tissue from the same part of the rats in each group, 5cm away from the terminal ileum, was repeatedly enema with ice normal saline, and cut into 1.5 CN large sections, half of which were placed in 4% paraformaldehyde. HE staining was used to detect the pathological changes of small intestinal tissue in each group. The degree of injury was evaluated according to the Aurelie Le Mandat Schultz pathological score. The score criteria were as follows: Small intestine epithelial tissue structure is normal and small intestine mucosal villus integrity was 0 points; small intestinal villus occurred slight edema, a small amount of epithelial cell degeneration and vacuolar changes was 1 point; Small intestinal villi were slightly broken and shed, and the degeneration of small intestinal epithelial cells was 2 points. In the small intestine, some intestinal villi were deficient, and leukocyte infiltration was 3 points. The damage of intestinal mucosal epithelial structure or transmural necrosis was 4 points.
- Another 1cm small intestinal tissue from the same part of the rats in each group, 7cm away from the end of the ileum, was frozen in liquid nitrogen. Fluorescence quantitative PCR and western blot were used to detect the mRNA and protein expression levels of BCL-2 and Bax in the small intestinal tissue of rats in each group.
- The small intestine tissues (2cm) at the same location (8cm away from the terminal ileum) of rats in each group were frozen in liquid nitrogen and detected by terminal deoxynucleotide transferase-mediated dUTP Nick end labeling method (TUNEL method) and immunohistochemical method.
- Another 5cm small intestinal tissue was taken to detect the contents of lactic acid and MDA in rat mucosal tissue by spectrophotometer, and the level of MPO was detected by colorimetric method.
- Another 2cm of small intestinal tissue was taken for the detection of mitochondrial transmembrane potential in intestinal mucosal cells, and the operation was performed in strict accordance with the kit instructions. The value of red and green fluorescence was recorded, and the ratio of red and green fluorescence was calculated to measure the change degree of mitochondrial transmembrane potential.

Statistical methods

SPSS22.0 software package was used for statistical data analysis. All measurement data in accordance with normal distribution were expressed as ($\bar{x}\pm s$), one-way analysis of variance was used for comparison between multiple groups, and SNK-Q test was used for pairwise comparison. Enumeration data were expressed as percentages, and comparison between groups was performed by chi-square test. $P<0.05$ was considered statistically significant.

Results

Comparison of pathological scores and pathological changes of rats in each group

The pathological scores of rats in the model group and Eparltast group were significantly

higher than those in the sham group (P<0.05). The pathological score of Eparltast group was significantly higher than that of the model group (P<0.05). See Table 1. In the sham-operation group, the structure of small intestinal epithelial tissue was basically normal, the structure of small intestinal epithelial tissue was normal, and the villi of small intestinal mucosa were intact, and no obvious pathological changes such as swelling, degeneration and necrosis occurred. In the model group, the small intestinal epithelial cells were necrotic, some villi were broken and shed, and the small intestinal epithelial structure was partially disappeared, and leukocyte infiltration was observed.

The pathological changes of small intestinal epithelium in Eparltast group were more serious.

Group	n	Pathological scores
Sham operation group	10	0.00
Model group	10	2.25±0.26[a]
Eparltast group	10	3.24±0.39[ab]
t		51.01
P		0.001

Table 1: Comparison of pathological scores of rats in each group (x̄±s).

Note: A represents compared with sham operation group, P<0.05; B represents compared with the model group, P<0.05.

Comparison of lactate, MDA and MPO levels in mucosal tissues of rats in each group

The lactate, MDA and MPO of mucosal tissue in model group and Eparltast group were significantly higher than those in the sham operation group (P<0.05). The lactate, MDA and MPO of mucosal tissue in Eparltast group were significantly higher than those in the model group (P<0.05). Are shown in Table 2.

Group	n	Lactic acid (mmol/g)	MDA (μmol/g)	MPO(U/g)
Sham operation group	10	2.76±0.21	6.24±0.54	0.09±0.02
Model group	10	5.61±0.53[a]	11.65±0.88[a]	0.48±0.03[a]
Eparltast group	10	7.52±0.67[ab]	15.27±1.02[ab]	0.65±0.03[ab]
t		9.66	210.28	124.09
P		0.008	0.001	0.001

Table 2: Comparison of lactic acid, MDA and MPO levels in mucosal tissues of rats in each group (x̄±s).

Note: A represents compared with sham operation group, P<0.05; B represents compared with the model group, P<0.05.

Comparison of apoptosis rate of intestinal mucosal cells in each group

The apoptosis rate of mucosal tissue in the model group and Eparltast group was significantly higher than that in the sham operation group (P<0.05). The apoptosis rate of mucosal tissue in Eparltast group was significantly higher than that in model group (P<0.05). See Table 3.

Group	n	Apoptosis (%)
Sham operation group	10	3.51±0.12
Model group	10	41.65±5.62[a]
Eparltast group	10	62.57±5.83[ab]
t		410.17
P		0.001

Table 3: Comparison of apoptosis rate of intestinal mucosa cells in each group.

Note: A represents compared with sham operation group, P<0.05; B represents compared with the model group, P<0.05.

Comparison of mRNA and protein expression levels of Bcl-2 and Bax in intestinal mucosal tissues of rats in each group

The mRNA and protein levels of Bcl-2 in mucosal tissues of the model group and Eparltast group were significantly lower than those of sham operation group (P<0.05). The mRNA and protein levels of Bcl-2 in mucosal tissues of rats in Eparltast group were significantly lower than those in the model group (P<0.05).

The mRNA and protein of Bax in mucosal tissues of the model group and Eparltast group were significantly higher than those of sham operation group (P<0.05). The mRNA and protein levels of Bax in mucosal tissues of rats in Eparltast group were significantly higher than those in the model group (P<0.05). Shown in Table 4.

		Bcl-2		Bax	
Group	n	mRNA	Protein	mRNA	Protein
Sham operation group	10	0.32±0.03	0.12±0.05	0.05±0.01	0.14±0.02
Model group	10	0.16±0.04[a]	0.07±0.02[a]	0.42±0.04[a]	0.49±0.10[aa]
Eparltast group	10	0.10±0.02[ab]	0.05±0.02[ab]	0.61±0.12[ab]	0.65±0.13[ab]
t		133.79	11.82	151.12	74.76
P		0.001	0.002	0.001	0.001

Table 4: Comparison of mRNA and protein expression levels of Bcl-2 and Bax in intestinal mucosal tissues of rats in each group (x̄±s).

Note: A represents compared with sham operation group, P<0.05; B represents compared with the model group, P<0.05.

Comparison of mitochondrial transmembrane potential of intestinal mucosal cells in each group

The mitochondrial membrane potential of rat mucosal tissue cells in model group and Eparltast group was significantly lower than that in the sham operation group (P<0.05). The mitochondrial membrane potential of rat mucosal tissue cells in

Eparltast group was significantly lower than that in model group (P<0.05). As shown in Table 5.

Group	n	Red fluorescence value	Green fluorescence value	Ratio
Sham operation group	10	24.68±1.42	7.92±0.60	3.54±0.24
Model group	10	19.47±1.08[a]	20.58±1.33[a]	0.95±0.11[a]
Eparltast group	10	15.36±1.21[ab]	25.61±1.62[ab]	0.72±0.08[ab]
t		140.85	524.39	966.71
P		0.001	0.001	0.01

Table 5: Changes of mitochondrial membrane potential in intestinal mucosal cells of rats in each group.
Note: A represents compared with sham operation group, P<0.05; B represents compared with the model group, P<0.05.

Discussion

As a member of the NADPH-dependent aldehyde-keto reductase family, aldose reductase is expressed in brain, nerve, kidney, retina, mucosa and other tissues, and is a key rate-limiting enzyme in the polyol pathway, which is involved in catalyzing the conversion of reduced aldehydes into corresponding alcohols[7]. As for the role of AR in ischemia-reperfusion injury, most of the mechanisms have been studied in myocardium and nerves[8].

Previous studies have found that the degree of myocardial injury in transgenic rats with high expression of AR is significantly higher than that in normal controls, and the reduction of AR expression can significantly alleviate myocardial ischemia-reperfusion injury and effectively improve myocardial energy metabolism[9]. Through the study of its mechanism in myocardial ischemia-reperfusion, it was found that AR may cause myocardial energy metabolism dysfunction by regulating glycolytic metabolic pathway, thereby increasing the concentration of Na^+ and $Ca2^+$ ions in cardiomyocytes and promoting cardiomyocyte injury[10]. However, other studies have obtained different results, suggesting that AR may play a protective role in inhibiting myocardial ischemia-reperfusion by ischemic preconditioning[11]. Studies have shown that ischemic preconditioning can effectively inhibit myocardial cell injury caused by myocardial ischemia-reperfusion, and the expression of AR is increased at the same time. After the addition of AR inhibitor, its protective effect disappeared, which subsequently proved that AR has a protective effect on myocardial cells after ischemia-reperfusion[12]. Subsequent studies have proved that the mechanism of its protective effect may be related to its REDOX characteristics[13]. How does oxidative stress play a role in ischemia-reperfusion intestinal mucosa? Existing studies have found that in the early stage of ischemia, xanthine dehydrogenase and adenylate in intestinal mucosa are converted into xanthine oxidase and hypoxanthine respectively, while in the perfusion period, with the increase of oxygen concentration, hypoxanthine and oxygen molecules generate xanthine under the action of xanthine oxidase, accompanied by the formation of a large number of ROS[14].

ROS can act on the unsaturated fatty acids of biofilms and form complexes through lipid peroxidation, thereby affecting the biological function of biofilms and leading to the loss of cell function or even death[15]. The results of this study showed that lactate, MDA and MPO in mucosal tissue of rats in model group and Eparltast group were significantly higher than those in the sham operation group (P<0.05). Lactate, MDA and MPO in the mucosal tissue of rats in Eparltast group were significantly higher than those in model group, indicating that AR expression was inhibited when the AR inhibitor epalrestat was added, and the intestinal mucosa injury was more serious with the increase of MDA content. The level of MDA can effectively reflect the level of lipid peroxides in the body and indicate the current oxidative stress state of the body. It can be seen that the mechanism by which AR plays the intestinal protective effect of ischemia-reperfusion is to reduce the oxidative stress level of the body through AR. As a reductase that catalyzes the conversion of aldehydes into alcohols, AR can be activated by hydrogen peroxide and aldehydes, effectively inhibiting apoptosis caused by oxidative stress[16]. On the contrary, when its expression is inhibited, vascular smooth muscle cells will cause cell death due to the increase of aldehyde content[17]. It can be seen that AR plays an antioxidant role by inhibiting oxidative stress.

Previous studies have found that inhibition of AR can effectively protect the key to platelet mitochondrial function; on the contrary, activation of AR can aggravate platelet mitochondrial dysfunction under ischemia-reperfusion[18]. This study found that the apoptosis rate of rat mucosal tissues in the Eparltast group was significantly higher than that in the model group, the mRNA and protein of Bcl-2 in the mucosal tissues of rats in the Eparltast group were significantly lower than those in the model group, and the mRNA and protein of Bax in the mucosal tissues of rats in the Eparltast group were

significantly higher than those in the model group. Moreover, the mitochondrial membrane potential of rat mucosal tissue cells in Eparltast group was significantly lower than that in model group. As the main apoptosis pathway, the mitochondrial pathway is involved in mediating intestinal mucosal barrier dysfunction, and the apoptosis index is correlated with the degree of intestinal function injury(19). It can be seen that the increase of intestinal mucosal apoptosis promotes the degree of intestinal ischemia-reperfusion mucosal injury. Existing studies have found that changes in mitochondrial transmembrane potential are considered to be the initial stage of mitochondrial structural and functional abnormalities, thereby mediating the apoptotic cascade(20). The ratio of Bcl-2/Bax was a key factor to determine the inhibitory effect on apoptosis.

In this study, the ratio of Bcl-2/Bax in mucosal tissues of rats in the model group and Eparltast group was significantly lower than that in the sham operation group ($P<0.05$). The ratio of Bcl-2/Bax in mucosal tissue of rats in Eparltast group was significantly lower than that in model group. These results suggest that AR inhibition may be involved in regulating the change of mitochondrial transmembrane potential, promote the apoptosis of intestinal mucosal cells, aggravate the damage of intestinal mucosa and affect the function of intestinal mucosa by regulating the Bcl-2/Bax ratio of intestinal mucosal cells.

In conclusion, aldose reductase plays a role in mediating acute hypoxic intestinal mucosal injury in young rats, and its mechanism may be realized by regulating the Bcl-2/Bax ratio in intestinal mucosal cells and regulating mitochondrial transmembrane potential.

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